Activation of signal transducer and activator of transcription 3 correlates with cell proliferation and renal injury in human glomerulonephritis

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Abstract

Background. Signal transducer and activator of transcription (STAT) 3 plays an important role in the regulation of cell proliferation. However, the mechanism of STAT3 activation in human glomerulonephritis is unclear.

Methods. STAT3 activation was determined using immunohistochemistry for phosphorylated STAT3 (p-STAT3) in normal human kidney and various types of glomerulonephritis. We also identified the cell exhibiting activated p-STAT3 expression in human glomerulonephritis and correlated STAT3 activation with renal function and histologic injury.

Results. p-STAT3 staining was identified in glomeruli and some tubules in normal human kidney. p-STAT3 positive glomerular cells were significantly increased in lupus nephritis, IgA nephropathy and vasculitis compared with normal kidney. p-STAT3 positive tubulointerstitial cells were significantly increased in IgA nephropathy and vasculitis compared with normal kidney. Glomerular and tubulointerstitial p-STAT3 staining was significantly decreased after steroid therapy. There was a significant correlation between the number of p-STAT3 positive cells and the number of PCNA positive glomerular and tubulointerstitial cells in all cases of glomerulonephritis. Furthermore, renal function inversely correlated with the number of p-STAT3 positive glomerular and tubulointerstitial cells in all cases of glomerulonephritis.

Conclusions. The present study has identified STAT3 activation in normal human kidney and a marked increase in STAT3 activation in many forms of glomerulonephritis. The correlation of STAT3 activation with clinical and histologic parameters suggests that this pathway plays an important role in the pathogenesis of kidney disease. Furthermore, localization of STAT3 activation to individual cell types suggests that this pathway may play a pivotal role in promoting renal inflammation and fibrosis.

Keywords: cell proliferation; glomerulonephritis; renal injury; STAT3

Introduction

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, is a well-described and important signal transduction cascade. STATs are a class of transcriptional regulatory proteins that are involved in the regulation of transcriptional activation [1]. The current model of JAK-STAT signalling is that engagement of the cytokine receptor activates the associated JAK. The JAK then phosphorylates the receptor cytoplasmic domain thereby facilitating the recruitment of a STAT that then undergoes dimerization following phosphorylation and translocates to the nucleus to bind specific sequences in the genome and activate gene expression [2].

STAT3 is a member of the JAK-STAT signalling pathway [3]. Cytoplasmic STAT3 of unstimulated cells is activated by recruitment through the SH2 domain to phosphotyrosine motifs within complexes of cytokine receptors (e.g. IL-6 receptor), growth factor receptors (e.g. epidermal growth factor receptor) or non-receptor tyrosine kinases (e.g. Src and Bcr-Abl). The biological functions of STAT3 are very broad. STAT3 plays a crucial role in the regulation of cell proliferation, survival, apoptosis and differentiation [4]. In fact, in vivo functional analyses using knockout mice indicate that STAT3 is required for embryogenesis because homozygous deletion of STAT3 results in early embryonic lethality. Furthermore, conditional knockout mice have demonstrated the pleiotropic role of STAT3 in many organs and cell types including the heart, skin, T lymphocytes, monocytes/neutrophils, mammary epithelium, liver and neurons [5].
STAT3 is activated by various stimuli. Previous in vitro studies indicated that STAT3 in mesangial cells was activated by angiotensin II [6] and high glucose [7] whilst STAT3 in proximal tubule cells was activated by interleukin 6 [8], high glucose [9] and oxidant stress [10]. In animal models, STAT3 activation has been reported in Anti-Thy 1-1 glomerulonephritis [11], fosinopril-induced immune complex glomerulonephritis [12], streptozotocin (STZ)-induced diabetes [13] and experimental ischaemia-reperfusion injury [14]. We also recently reported that STAT3 is activated in the model of unilateral ureteral obstruction (UUO) [15]. In addition, we reported that mesangial cell STAT3 is activated by a platelet-derived growth factor (PDGF) in vitro whilst PDGF receptor tyrosine kinase inhibitor suppression of mesangial cell proliferation involves STAT3 activation in vivo and in vitro [16]. Despite these in vitro data and studies in experimental models, the mechanism of STAT3 activation in human glomerulonephritis is currently unclear.

The aim of this study was to investigate the potential role of STAT3 activation in the pathogenesis of human glomerulonephritis. We examined STAT3 activation by immunohistochemical staining of renal biopsies from a broad cross-section of glomerulonephritis. We identified the cell type exhibiting phosphorylated STAT3 (p-STAT3) pathway activation in human glomerulonephritis and correlated STAT3 activation with clinical parameters of renal function and histological injury.

Subjects and methods

Patients
Renal biopsies were performed at Hiroshima University Hospital for diagnostic purposes in accordance with best clinical practice, and informed consent for the use of renal biopsy tissue, in excess of that required for diagnostic purposes, was obtained from the patients. Renal biopsies from 45 patients were analysed. Renal biopsies were performed in all 45 cases before treatment. Disease categories were based on histological examination of biopsy specimens. The classification of human renal diseases and clinical parameters are given in Table 1. We performed a second renal biopsy after treatment with steroids in eight patients. An immunosuppressive drug was used in addition to steroid therapy in one of the eight cases whilst angiotensin II receptor blockers (ARBs) were administered in two of the eight cases. In addition, renal tissues from an uninvolved pole of five carcinoma nephrectomy specimens were used as normal controls.

Antibodies
The following antibodies and reagents were used in this study: anti-phosphorylated (Tyr705)-STAT3 (p-STAT3) rabbit monoclonal antibody (mAb) (Cell Signaling Technology, Beverly, MA, USA); phosphorylated (Try705)-STAT3 peptide (Cell Signaling Technology); anti-STAT3 antibody (Cell Signaling Technology); PC 10, anti-proliferating cell nuclear antigen (PCNA) mouse mAb, which recognizes cells in G1, S and G2 phases of cell cycle (Dako, Glostrup, Denmark); KP1, anti-human CD68 mouse mAb (Dako); 1A4, anti-smooth muscle actin (α-SMA), recognizing human myofibroblasts (Sigma-Aldrich, St. Louis, MO, USA). The following secondary polyclonal antibodies and reagents were used in this study: biotinylated goat anti-rabbit IgG (Zymed, San Francisco, CA, USA); avidin–biotin complexes (ABC) (Vector Laboratories, Burlingame, CA, USA); horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Dako) and peroxidase-conjugated mouse anti-peroxidase (PAP) complexes (Dako).

Immunohistochemistry
Renal biopsy tissues were fixed in 10% formalin, washed in ethanol and embedded in paraffin. Four-micrometre thick sections were used for immunohistochemistry. Detection of p-STAT3 and STAT3 used the ABC method. Paraffin sections of formalin-fixed tissues were dewaxed in xylene, rehydrated and heated in a microwave oven in 0.1 M sodium citrate for 15 min. The sections were then washed in PBS, blocked with 10% goat serum for 1 h and incubated with 1:50 dilution of anti-p-STAT3 rabbit mAb and with 1:100 dilution of the anti-STAT3 antibody in 5% normal human serum overnight at 4°C. The sections were then washed and endogenous peroxidase activity blocked by incubation in 0.6% H2O2 in methanol for 20 min. They were washed in PBS and avidin and biotin blocks were applied (Vector Laboratories). After washing in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG for 45 min, washed and incubated with the ABC complex for 45 min.

Table 1. Classification and clinical parameters of patients with glomerulonephritis

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Age (years)</th>
<th>Gender (M:F)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Proteinuria (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>67.4 ± 6.4</td>
<td>5:0</td>
<td>14.4 ± 1.9</td>
<td>0.85 ± 0.11</td>
<td>95.4 ± 30.8</td>
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<tr>
<td>MCD</td>
<td>9</td>
<td>38.1 ± 17.7</td>
<td>6:3</td>
<td>16.3 ± 5.5</td>
<td>0.85 ± 0.31</td>
<td>95.7 ± 28.3</td>
</tr>
<tr>
<td>MG2</td>
<td>10</td>
<td>61.7 ± 14.1</td>
<td>8:2</td>
<td>15.2 ± 5.8</td>
<td>0.84 ± 0.19</td>
<td>83.9 ± 24.4</td>
</tr>
<tr>
<td>IgA</td>
<td>10</td>
<td>29.8 ± 13.7</td>
<td>6:4</td>
<td>12.9 ± 3.7</td>
<td>0.79 ± 0.17</td>
<td>93.8 ± 14.3</td>
</tr>
<tr>
<td>Lupus III/IV</td>
<td>9</td>
<td>33.2 ± 9.7</td>
<td>1:8</td>
<td>14.8 ± 6.1</td>
<td>0.79 ± 0.25</td>
<td>95.0 ± 21.6</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>7</td>
<td>64.7 ± 19.2</td>
<td>3:4</td>
<td>38.7 ± 30.4*</td>
<td>2.02 ± 1.35*</td>
<td>42.9 ± 47.9*</td>
</tr>
</tbody>
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MCD, minimal-change disease; MG, membranous glomerulonephritis; IgA, IgA nephropathy; Lupus III/IV, lupus nephritis International Society of Nephrology/Renal Pathology Society (ISN/RPS) class III/IV; vasculitis, pauci-immune segmental necrotising crescentic glomerulonephritis.

Data are presented as the mean ± SD. *P < 0.05 compared with normal.
at room temperature. After washing in PBS, the sections were developed with 3,3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) to produce a brown colour. Specificity of p-STAT3 immunostaining was demonstrated by abrogation of the staining pattern by pre-incubation of the anti-p-STAT3 antibody with twice the volume of p-STAT3 blocking peptide for 30 min at room temperature prior to incubation of the sections. The sections underwent antigen retrieval by heat-incubation of the sections.

Blocking of the sections was performed with the p-STAT3 antibody with brown colour and staining. For two-colour immunostaining, the sections were stained with the p-STAT3 antibody with brown colour and staining. For two-colour immunostaining, the sections were stained with the p-STAT3 antibody with brown colour and then heated in a microwave oven to prevent antibody cross-reactivity [17]. The sections were then stained with the α-SMA and CD68 antibodies with blue-grey colour development.

All 50 sections were immunostained for p-STAT3 and PCNA. However, because of limitations in tissue availability, double staining for p-STAT3/α-SMA and p-STAT3/CD68 was performed on three cases each of IgA nephropathy, class III/IV lupus nephritis and vasculitis whilst immunostaining of STAT3 was performed on three cases each of normal kidney, IgA nephropathy and vasculitis.

Quantification of p-STAT3 staining

All tissues examined contained at least 10 glomeruli that did not exhibit sclerosis. The numbers of p-STAT3 and PCNA immunostained cells (one-colour staining), including cells within glomerular crescents, were counted and the data presented as the mean cell number ± SD per glomerular cross-section. The numbers of tubular and interstitial p-STAT3 positive cells were counted in five high-power (×400) fields (avoiding glomeruli and large vessels) and the data expressed as the mean number of positive p-STAT3 cells ± SD per mm². All counting was performed by observers who were blinded to the renal diagnosis.

Quantification of histological injury

The total number of glomeruli and the number of globally sclerosed or crescentic glomeruli were counted. The number of nuclei per glomerular cross-section (including crescents) was also counted. The degree of interstitial cell infiltration and the degree of interstitial fibrosis or tubular atrophy were scored between 0 and 3+ according to the following criteria: 0 = 0 to 5%; 1+ = 6 to 20%; 2+ = 21 to 50%; and 3+ ≥ 50% of the biopsy area demonstrated infiltration or fibrosis (Table 2).

Statistical analyses

Data were presented as mean ± SD. All analysis was performed using StatView 5.0 (SAS institute Inc., Cary, NC, USA). The treatments and post-treatment groups were compared using the paired t-test. Other comparisons between groups were made by one-way ANOVA using Dunnett multiple comparisons. The Pearson single correlation analysis was used to determine the correlation between the number of glomerular and interstitial p-STAT3 positive cells and renal function, proteinuria and glomerular cellularity. The Spearman correlation analysis was used to compare the number of glomerular and interstitial p-STAT3 positive cells with the percentage of sclerosed or crescentic glomeruli, the degree of interstitial cell infiltration and the degree of interstitial fibrosis or tubular atrophy.

Results

STAT3 activation in normal human kidney

Normal human kidney derived from an uninvolved pole of carcinoma nephrectomy samples exhibited small numbers of p-STAT3 positive glomerular cells (Figures 1a and 2a). Small numbers of tubular epithelial cells and occasional interstitial cells were p-STAT3 positive (Figures 1b and 2b). STAT3 activation was seen in all tubular segments and collecting ducts with no clear restriction to any one particular region evident.

STAT3 activation in human glomerulonephritis

In comparison with normal kidney, there was a significant increase in the number of p-STAT3 positive glomerular cells in lupus nephritis (International Society of Nephrology/Renal Pathology; ISN/RPS class III/IV) and IgA nephropathy (Figure 1c) with p-STAT3 positive cells...
Activation of STAT3 correlates with renal injury, being particularly prominent in pauci-immune crescentic glomerulonephritis (vasculitis) (Figure 1e). In contrast, there was no increase evident in non-proliferative glomerulonephritis such as minimal-change disease (MCD) and membranous glomerulonephritis (MGN) (Figure 2a). There were also many p-STAT3 positive cells within glomerular crescents (Figure 1c).

The numbers of tubular and interstitial p-STAT3 positive cells were significantly increased in IgA nephropathy (Figure 1d) and vasculitis (Figure 1f) compared with normal kidney (Figure 2b). However, in lupus nephritis, there was no significant increase evident in p-STAT3 staining. There was no increase of p-STAT3 staining in non-proliferative glomerulonephritis (MCD and MGN). The greatest increase in the number of p-STAT3 positive cells was seen in vasculitis. The increased p-STAT3 expression within tubules involved all tubular segments including the proximal and distal convoluted tubules and collecting ducts.

The specificity of p-STAT3 immunostaining was demonstrated by abrogation of the staining pattern by controls in which the p-STAT3 antibody was incubated with the p-STAT3 blocking peptide before being applied to the tissue section (Figure 1g).

**STAT3 expression in normal human kidney and glomerulonephritis**

Staining of STAT3 in normal human kidney is shown in Figure 1h. Many STAT3 positive cells were seen in the glomeruli and tubulointerstitium of normal kidney. There was no increase in the number of STAT3 positive cells in the glomeruli and tubulointerstitium of IgA nephropathy (data not shown) and vasculitis (Figure 1i) compared to normal kidney tissue.

**Cell proliferation in normal human kidney and glomerulonephritis**

Cell proliferation was determined by counting glomerular cells following immunostaining for PCNA. There was an increase in the glomerular cell number in proliferative glomerulonephritis (IgA nephropathy, lupus nephritis and vasculitis) compared with normal kidney (Table 2).

In normal human kidney, there were small numbers of PCNA positive cells in glomeruli (Figure 3a) and small...
numbers of tubular epithelial cells, and occasional interstitial cells were p-STAT3 positive (data not shown). In the glomeruli, the number of PCNA positive cells was increased in vasculitis (Figures 3c and Figure 4a) with PCNA positive cells being particularly prominent within cellular crescents. Indeed, the majority of glomerular p-STAT3 and PCNA positive cells were present within crescents. There was no significant increase in PCNA staining in IgA nephropathy (3b) and lupus nephritis. There was no increase in the number of PCNA positive cells in MCD and MGN. The numbers of tubular and interstitial PCNA positive cells were increased in vasculitis (Figures 3d and 4b) but there was no significant increase evident in any other glomerulonephritis.

Localization of p-STAT3 in human glomerulonephritis

Double staining of p-STAT3 and macrophage (CD68) in vasculitis is shown in Figure 5a and b. Many p-STAT3 positive cells and macrophages were seen in glomeruli, tubules, and interstitium of vasculitis. Many p-STAT3 positive cells are also located in the tubules and interstitium of MGN, MCD, and IgA nephropathy. There was no significant increase evident in any other glomerulonephritis.

**Fig. 3.** Glomerular and tubulointerstitial proliferating cell nuclear antigen (PCNA) expression in normal and diseased human kidney. Serial section to Figure 1(a) showing little PCNA staining in glomeruli of normal kidney (a). Serial section to Figure 1(c) showing PCNA staining in the mesangial area of IgA nephropathy (b). Serial section to Figure 1(e) showing many PCNA positive cells in the glomeruli and crescents of vasculitis (c). Serial section to Figure 1(f) showing many PCNA positive tubular and interstitial cells in a case of vasculitis (d). Original magnification ×200. Bar, 20 µm.

**Fig. 4.** Quantification of PCNA immunostaining in normal and diseased human kidney. Immunostained tissue sections are scored for the number of (a) PCNA positive cells per gcs and (b) PCNA positive tubular cells per mm². For each disease classification, data are presented as the mean ± SD. *P < 0.05 versus normal.

**Fig. 5.** Two-colour immunostaining of p-STAT3 in human glomerulonephritis. Vasculitis showing double staining for p-STAT3 (brown) and macrophages (CD68, blue-grey cytoplasmic staining). Many p-STAT3 positive cells are seen in glomeruli (G), crescents (C) and the interstitium (a). High power of (a) demonstrating some p-STAT3 and CD68 double stained cells in a crescent (C) and the interstitium (arrows) (b). A vasculitis biopsy showing double staining for p-STAT3 (brown) and alpha smooth muscle actin (α-SMA, blue-grey). Many p-STAT3 positive cells are seen in the tubules and the interstitium. Also, many infiltrating myofibroblasts (α-SMA) are seen in an area of interstitial fibrosis (c). High power photomicrograph (c) demonstrating significant p-STAT3 staining of interstitial α-SMA myofibroblasts (arrows) (d). Original magnification, ×200 (a, c) and ×400 (b, d). Bar, 20 µm.
Activation of STAT3 correlates with renal injury

Correlation of the number of p-STAT3 positive cells with the number of PCNA positive cells. Analysis of all patients as one cohort by the Pearson correlation coefficient indicated a significant correlation between the number of glomerular p-STAT3 positive cells and PCNA positive cells (a), and between the number of tubular p-STAT positive cells and PCNA positive cells (b).

Correlation of STAT3 activation with cell proliferation

Correlation of STAT3 activation with clinical parameters and histological parameters of renal injury in human glomerulonephritis

The clinical parameters of patients with glomerulonephritis and the histological parameters of renal injury are shown in Table 3. There was a positive correlation between the serum creatinine concentration and the number of glomerular and interstitial p-STAT3 positive cells in all cases of glomerulonephritis. Creatinine clearance inversely correlated with the number of glomerular and interstitial p-STAT3 positive cells in all cases of glomerulonephritis. However, the 24-h urinary protein excretion did not correlate with the number of glomerular and interstitial p-STAT3 positive cells.

Regarding the histological parameters, there was a significant correlation between the number of p-STAT3 positive glomerular cells and glomerular cellularity. There was also a significant correlation between the number of p-STAT3 positive glomerular cells and the percentage of sclerotic glomeruli with crescents. The number of p-STAT3 positive interstitial cells correlated with the degree of interstitial inflammation and the degree of interstitial fibrosis or tubular atrophy.

However, the percentage of sclerotic glomeruli did not correlate with the number of p-STAT3 glomerular positive cells.

STAT3 activation in glomerulonephritis before and after steroid treatment

We performed a second renal biopsy after treatment with steroids in eight cases of 45 patients: three cases of IgA nephritis, two cases of Lupus nephritis and three cases of vasculitis. In these eight cases, we compared STAT3 activation and PCNA expression in biopsies performed before and after steroid treatment. There was a significant decrease in the number of glomerular and tubulointerstitial p-STAT3 positive cells (Figure 7a and b) of post-treatment biopsies compared to pre-treatment biopsies. In addition, there was a significant decrease in the number of PCNA positive cells in the tubulointerstitium (Figure 7d) but not in the glomeruli (Figure 7c) of post-treatment biopsies.

Discussion

To our knowledge, this is the first study to examine STAT3 activation in normal and diseased adult human kidney. In this study, p-STAT3 staining was identified in glomeruli and some tubules in normal human kidney. The stimulus for STAT3 activation in mesangial and tubulointerstitial cells found in normal human kidney is unclear. The glomerular and tubulointerstitial p-STAT3 staining found in normal human kidney is consistent with studies in normal rat kidney [16]. The limited presence of p-STAT3 in normal renal tissue suggests that limited STAT3 activation might not play a critically important role in normal human renal physiology.

We previously reported that PDGF activated STAT3 in mesangial cells in vitro and that a PDGF receptor tyrosine kinase inhibitor suppressed mesangial cell proliferation involving STAT3 activation in vivo and in vitro [16]. We also reported that STAT3 was activated in rat tubular epithelial cells and myofibroblast in the experimental model of...
Table 3. Correlation analysis of p-STAT3 activation with clinical and pathological disease parameters

<table>
<thead>
<tr>
<th></th>
<th>Serum creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Proteinuria (g/day)</th>
<th>Glomerular cellularity (cells/gcs)</th>
<th>Sclerosis (%)</th>
<th>Crescents (%)</th>
<th>Interstitial cell infiltration (0–3+)</th>
<th>Interstitial fibrosis or tubular atrophy (0–3+)</th>
</tr>
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<tbody>
<tr>
<td>p-STAT3 Positive Glomerular cells (gcs)</td>
<td>0.61</td>
<td>−0.59</td>
<td>−0.15</td>
<td>0.66</td>
<td>0.21</td>
<td>0.82</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>NS</td>
<td>(NS)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td></td>
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<tr>
<td>p-STAT3 Positive Tubular cells (mm²)</td>
<td>0.70</td>
<td>−0.60</td>
<td>−0.04</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.73</td>
<td>0.48</td>
</tr>
<tr>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>NS</td>
<td>(NS)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
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All patients with glomerulonephritis were analysed as a single cohort. Data are shown as the correlation coefficient r and statistical values given in parenthesis. NS represents ‘not significant’.

Fig. 7. Quantification of p-STAT3 and PCNA immunostaining in diseased human kidney before and after treatment with steroids. The before and after treatment groups include eight specimens derived from patients who underwent a renal biopsy before and after treatment with steroids. Immunostained tissue sections were scored for the number of (a) p-STAT3 positive cells per glomerular cross-section (gcs); (b) p-STAT3 positive tubular cells per mm²; (c) PCNA positive cells per gcs and (d) PCNA positive tubular cells per mm². For each disease classification, data are presented as the mean ± SD. *P < 0.05

UUO [15]. STAT3 activation in glomeruli was reported in models of proliferative glomerulonephritis [11–14] whilst STAT3 activation in the tubulointerstitium was reported in the model of fosinopril-induced immune complex glomerulonephritis [12]. In the present study, glomerular p-STAT3 staining was not increased in MCD and MGN compared with normal kidney. In contrast, there was a significant increase in proliferative glomerulonephritis (IgA nephropathy, lupus nephritis and vasculitis). In the tubulointerstitium, p-STAT3 staining significantly increased in vasculitis and the STAT3 activation in human glomerulonephritis found in the present study is consistent with previous animal studies. In addition, we found that the glomerular and interstitial expression of STAT3 is almost constant in normal kidney and glomerulonephritis and not associated with the degree of histological damage. These results indicate that upregulation of p-STAT3 is due to increased activation of STAT3 by various stimuli, and is not secondary to increased levels of total STAT3 including unactivated STAT3.

Mesangial cell proliferation is a prominent feature of glomerular diseases including IgA nephropathy, lupus nephritis and some types of steroid resistant nephrotic syndrome as well as other lesions [18]. We found a strong correlation between STAT3 activation and glomerular cell proliferation in this study. We previously reported a correlation between STAT3 activation and mesangial cell proliferation in the model of rat Thy-1-1 glomerulonephritis [16]. Tubular cell activation and interstitial cell infiltration stimulate fibroblast proliferation through the secretion of cytokines and growth factors leading to tubulointerstitial fibrosis [19]. It is therefore noteworthy that we also found a strong correlation between STAT3 activation and tubulointerstitial cell proliferation in this study. We also reported a correlation between STAT3 activation and proliferation of tubular epithelial cells and interstitial cells in the rat model of UUO [15]. The correlations evident in the present study are consistent with the results of our previous animal studies. It has been previously reported that STAT3 activation
contributes to cell proliferation in various organs and we now demonstrate that STAT3 plays a similar role in human glomerulonephritis.

In diseased kidney, fibroblasts become activated, undergo dramatic proliferation and produce excessive extracellular matrix. During this process fibroblasts undergo functional and phenotypic changes acquiring a myofibroblastic phenotype, which ultimately correlates with the development of tubulointerstitial fibrosis [20]. The previous work indicates that myofibroblasts express STAT3 [21]. Double immunostaining of α-SMA and p-STAT3 demonstrated many double-positive fibroblasts exhibiting staining of α-SMA with p-STAT3. In addition, we found a strong correlation between interstitial STAT3 activation and interstitial fibrosis. In our previous study, we noted the presence of diffuse numbers of double-positive myofibroblasts within the interstitium of the obstructed rat kidney [15]. These results indicate that STAT3 activation plays an important role in the accumulation of interstitial myofibroblasts that ultimately results in interstitial fibrosis.

We used double immunostaining to examine which cells expressed increased p-STAT3 staining. Macrophage accumulation is a prominent feature of most types of human glomerulonephritis. In particular, tubulointerstitial macrophage accumulation correlates with the degree of renal dysfunction and is predictive of disease progression [22]. By using double immunostaining of CD68 and p-STAT3, we found a few double-positive macrophages. The previous in vitro work using human macrophages suggests that STAT3 is activated by IL-6 and IL-10 [23]. However, in this study, there were only a few cells that expressed both CD68 and p-STAT3, and this result concurs with our previous work [15,16]. This suggests that the STAT3 pathway might not play an important role in macrophage-mediated renal injury in human glomerulonephritis.

In this study, we found a correlation between glomerular and tubulointerstitial STAT3 activation and renal function indicating that, from the clinical perspective, STAT3 activation plays an important role in renal injury. However, there was no correlation between glomerular or tubulointerstitial STAT3 activation and proteinuria. Zhang et al. reported that glomerular STAT3 activation significantly correlated with proteinuria in the model of fosinopril-induced immune complex glomerulonephritis [12]. Although the reason for this unexpected discrepancy is unclear, it may simply reflect the fact that our study was performed in various forms of kidney disease and not in a more uniform experimental model. It also may reflect the transient nature of STAT3 activation before the proteinuric phase of chronic kidney disease.

We analysed renal biopsies before and after treatment with steroids in eight cases and found that the number of glomerular and tubulointerstitial p-STAT3 positive cells in post-treatment biopsies was significantly decreased compared to pre-treatment biopsies. Since we compared biopsies from the same patients before and after steroid treatment, these results suggest that steroid therapy contributes to the downregulation of p-STAT3.

In summary, this study has identified STAT3 activation in normal human kidney and a marked increase in STAT3 activation in many forms of glomerulonephritis. The correlation of STAT3 activation with clinical and histological parameters argues that this pathway plays an important role in disease pathogenesis. Furthermore, localization of STAT3 activation to individual cell types suggests that this pathway may play a pivotal role in promoting both renal inflammation and fibrosis. Thus, blockade of STAT3 may provide a novel approach in the treatment of human glomerulonephritis.

Conflict of interest statement. None declared.

References


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